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TITLE

Priming exercise accelerates pulmonary oxygen uptake kinetics during “work-to-work” cycle exercise in middle-aged individuals with type 2 diabetes.

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Abstract

Purpose The time constant of phase II pulmonary oxygen uptake kinetics ($\dot{V}O_{2\tau_p}$) is increased when high-intensity exercise is initiated from an elevated baseline (work-to-work). A high-intensity priming exercise (PE), which enhances muscle oxygen supply, does not reduce this prolonged $\dot{V}O_{2\tau_p}$ in healthy active individuals, likely because $\dot{V}O_{2\tau_p}$ is limited by metabolic inertia (rather than oxygen delivery) in these individuals. Since $\dot{V}O_{2\tau_p}$ is more influenced by oxygen delivery in type 2 diabetes (T2D), this study tested the hypothesis that PE would reduce $\dot{V}O_{2\tau_p}$ in T2D during work-to-work cycle exercise. **Methods** Nine middle-aged individuals with T2D and nine controls (ND) performed four bouts of constant-load, high-intensity work-to-work transitions, each commencing from a baseline of moderate-intensity. Two bouts were completed without PE and two were preceded by PE. The rate of muscle deoxygenation ([HHb+Mb]) and surface integrated electromyography (iEMG) were measured at the right and left vastus lateralis respectively. **Results** Subsequent to PE, $\dot{V}O_{2\tau_p}$ was reduced ($P=0.001$) in T2D (from 59 ± 17 to 37 ± 20 s) but not ($P=0.24$) in ND (44 ± 10 to 38 ± 7 s). The amplitude of the $\dot{V}O_2$ slow component ($\dot{V}O_{2A_s}$) was reduced ($P=0.001$) in both groups (T2D: 0.16 ± 0.09 to 0.11 ± 0.04 l/min; ND: 0.21 ± 0.13 to 0.13 ± 0.09 l/min). This was accompanied by a reduction in $\Delta iEMG$ from the onset of $\dot{V}O_2$ slow component to end-exercise in both groups ($P<0.001$), while [HHb+Mb] kinetics remained unchanged. **Conclusions** PE accelerates $\dot{V}O_{2\tau_p}$ in T2D, likely by negating the O_2 delivery limitation extant in the unprimed condition, and reduces the $\dot{V}O_{2A_s}$ possibly due to changes in muscle fibre activation.

Keywords: near-infrared spectroscopy, oxygen extraction, cycling, oxygen uptake slow component, electromyography.

Abbreviations:

A: Amplitude

CP: Critical power

HR: Heart rate

HHb+Mb: deoxygenated haemoglobin and myoglobin

iEMG: Surface integrated electromyography

MRT: Mean response time

ND: Non-diabetic controls

53	NIRS: Near-infrared spectroscopy
54	PE: Priming exercise
55	TD: Time delay
56	T2D: Type 2 diabetes
57	TOI: Tissue oxygenation index
58	$\dot{V}CO_2$: Expired carbon dioxide
59	\dot{V}_E : Minute ventilation
60	$\dot{V}O_2$: Oxygen uptake
61	$\dot{V}O_{2peak}$: Peak oxygen uptake
62	VT: Ventilatory threshold
63	w-to-w: Work-to-work transition
64	τ : time constant
65	$\Delta 50\%$: the sum of the power output at VT and 50% of the difference between the power output at VT and
66	$\dot{V}O_{2peak}$
67	$\Delta iEMG_{end-TDs}$: Difference between iEMG values at end-exercise and at the time point equivalent to the onset of
68	TD.
69	

Introduction

Type 2 diabetes mellitus (T2D), having reached epidemic proportions in the last two decades, poses one of the main threats to human health in the 21st century. Of significant concern for this clinical population is the consistent demonstration of a reduced maximal exercise capacity (Green et al. 2015), which is independently correlated with cardiovascular and all-cause mortality (Kodama et al. 2009). Furthermore, pulmonary oxygen uptake ($\dot{V}O_2$) kinetics during moderate-intensity (i.e. below ventilatory threshold, (VT)) exercise is significantly blunted by ~30% in young and middle-aged individuals with uncomplicated T2D (Mac Ananey et al. 2011; O'Connor et al. 2015; O'Connor et al. 2012; Kiely et al. 2015; Bauer et al. 2007; Regensteiner et al. 1998). This is evidenced by a prolonged time constant of the primary phase of the $\dot{V}O_2$ kinetics response ($\dot{V}O_2 \tau_p$) which has been considered to be a determinant of exercise tolerance (Jones and Poole 2005). Although not universal (Poitras et al. 2015; Copp et al. 2010), substantial evidence exists to suggest that the impairments in $\dot{V}O_2 \tau_p$ in uncomplicated T2D are influenced by limitations in peripheral oxygen (O_2) delivery in the lower limbs (Kiely et al. 2014; Bauer et al. 2007; MacAnaney et al. 2011). In contrast, in non-diabetic active individuals presenting with a fast $\dot{V}O_2$ kinetics, $\dot{V}O_2 \tau_p$ appears to be limited by the adjustment of specific metabolic pathways (i.e. oxidative capacity of contracting skeletal muscle) rather than O_2 delivery.

In healthy active individuals, the initiation of a transition to heavy-intensity [$> VT$ and $<$ critical power (CP)] or severe-intensity ($> CP$) upright cycling from a moderate-intensity ($< VT$) baseline, referred to as work-to-work (w-to-w), elicits a significantly longer $\dot{V}O_2 \tau_p$ than an on-transition from rest or 'unloaded' cycling (Hughson and Morrissey 1982; Goulding et al. 2018; Wilkerson and Jones 2007, 2006; DiMenna et al. 2009; DiMenna et al. 2008). This prolonged $\dot{V}O_2 \tau_p$ may relate to a constrained cellular respiration in the already active muscle fibres (Nederveen et al. 2017), or a larger recruitment of fast twitch (type II) muscle fibres to meet the augmented metabolic demand (Whipp 1994; Barstow et al. 1996). In these healthy active individuals a prior bout of high-intensity priming exercise (PE) does not alter this prolonged $\dot{V}O_2 \tau_p$ in subsequent w-to-w transitions. This is likely because during high-intensity exercise a prior PE appears to facilitate convective and diffusive components of muscle O_2 delivery (Gerbino et al. 1996; Sahlin et al. 2005; Jones et al. 2006) rather than muscle metabolic pathways. In this regard, DiMenna et al. (DiMenna et al. 2010b) reported that PE significantly reduced $\dot{V}O_2 \tau_p$ during severe-intensity w-to-w cycling in the supine posture, where O_2 delivery is limited due to a loss of gravity-enhanced perfusion pressure in active muscle. These effects were, therefore, likely owing to an enhanced distribution of blood flow to active muscles following priming (DiMenna et al. 2010b). Despite PE not influencing

$\dot{V}O_2 \tau_p$ during heavy/severe-intensity w-to-w upright cycling in healthy active participants, PE reduces the mean response time (MRT) of the overall $\dot{V}O_2$ dynamic response by increasing the amplitude of the $\dot{V}O_2$ primary phase ($\dot{V}O_2 A_p$) and/or blunting the amplitude of the $\dot{V}O_2$ slow component ($\dot{V}O_2 A_s$), which have been associated with a PE-induced reduction in the requirement for type II muscle fibre activation, and thus, an improved metabolic stability of type I fibres (DiMenna et al. 2008).

Given that T2D is a disease that affects the vasculature and limits the O_2 supply to contracting muscle, the combination of a PE intervention with the w-to-w model may offer further insight into potential mechanisms implicated in the impaired $\dot{V}O_2$ kinetics response demonstrated by these individuals. Accordingly, the aim of the present study was to investigate the influence of PE on $\dot{V}O_2$ kinetics during w-to-w upright cycling exercise transitions in middle-aged individuals with T2D. We hypothesized that PE would speed $\dot{V}O_2 \tau_p$ in the subsequent high-intensity w-to-w transition in individuals with T2D. Given that muscle fibre distribution appears to be altered in individuals with T2D (Marin et al. 1994) with reports showing a 2-fold increase in type IIb fibres (Mogensen et al. 2007), together with the notion that PE induces a reduction in type II muscle fibre activation, we also hypothesised that PE would reduce the $\dot{V}O_2 A_s$ in individuals with T2D. In attempting to explore the mechanistic basis of any PE-induced effect on $\dot{V}O_2$ kinetics in T2D, the rate of muscle deoxygenation (i.e., deoxygenated haemoglobin and myoglobin, HHb+Mb) and muscle electromyography (EMG) were measured to assess the alterations on muscle fractional O_2 extraction and motor unit activation, respectively.

Methods

Participants

Eighteen individuals, 9 with uncomplicated T2D (5 males/4 females) and 9 healthy controls (5 males/4 females) volunteered to participate in this study (Table 1). Non-diabetic controls (ND) were recruited from the general population, whilst participants with T2D were recruited from the Diabetes Outpatient Clinics of St. Columcille's Hospital (Loughlinstown, Co. Dublin) and St. Vincent's University Hospital (SVUH, Dublin 4) following chart review. To avoid the potential confounding effects of age on the T2D-related impairments in exercise tolerance, previously established in men (Wilkerson et al. 2011; O'Connor et al. 2015), we limited the age of participants to < 60 yr.

Three female participants were premenopausal (1 T2D and 2 ND) and five were postmenopausal (3 T2D and 2 ND) not undergoing hormone replacement therapy. Participants were classified as physically inactive by self-

report (≤ 1.5 h.week⁻¹ of moderate-intensity exercise in the preceding 6 months), which was confirmed by the use of 5-day RT3 triaxial accelerometry (Stayhealthy Inc, CA) in a subset of participants (Table 1) (Rowlands et al. 2004). All participants with T2D had a clinical history of diabetes between 3 and 11 years (mean \pm SD = 7.3 ± 4.0 yrs.), were treated by oral hypoglycaemic agents and had adequately controlled HbA_{1c} levels ($< 8.5\%$). None of the participants with T2D were taking insulin or beta-blockers and all participants were non-smokers (had not smoked during the 12-month period preceding the study). One of the healthy controls was on a prescriptive medication (statins, $n = 1$), and individuals with T2D were taking oral ($n = 8$) and/or subcutaneous ($n = 1$) hypoglycaemic prescription medications (metformin, $n = 5$; sulphonylurea, $n = 1$; glucagon-like peptide 1, $n = 1$; sodium glucose cotransporter-2 inhibitors, $n = 1$; dipeptidyl peptidase-4 inhibitors, $n = 1$). In addition, 3 individuals with T2D were taking antihypertensive prescription drugs (angiotensin converting enzyme inhibitor & calcium channel blocker, $n = 2$; angiotensin II receptor blocker, $n = 1$) and statins. All participants displayed no clinical evidence of coronary artery disease (12-lead electrocardiogram treadmill stress test following the Bruce protocol), peripheral arterial disease ($0.9 < \text{Ankle-Brachial Index, ABI}, < 1.3$), kidney dysfunction (consistent urinary protein > 200 mg.dl⁻¹) or liver dysfunction (urinary creatinine levels > 2.2 mg.dl⁻¹). All participants provided written informed consent prior to participation. The study was approved by the Faculty of Health Sciences' Research Ethics Committee, Trinity College Dublin, and St Vincent's Healthcare Ethics and Medical Research Committee, and was performed in line with the principles outlined by the Declaration of Helsinki.

Study Protocol

Overview. Following the satisfactory completion of the 12-lead ECG stress test, all participants completed two visits to the laboratory. The controls undertook these tests in the cardiovascular performance laboratory in the Department of Physiology, Trinity College Dublin; whilst individuals with T2D did so in the exercise testing facility in St. Columcille's Hospital. In the first visit all participants performed a ramp incremental (RI) cycling test to exhaustion to determine $\dot{V}O_{2\text{peak}}$ (see *visit 1*). In the second visit participants performed four w-to-w step transitions to high-intensity exercise commencing from a baseline of moderate-intensity exercise. Two of these transitions were completed without PE and the other two transitions were undertaken preceded by a PE (see *visit 2*). All exercise tests were carried out in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, Netherlands). All participants were asked to refrain from consuming alcohol, caffeine and non-prescribed nutritional supplements as well as avoiding any strenuous exercise in the 24 hours prior to testing. All premenopausal participants were tested during the mid-follicular phase (days 5-12) of the

menstrual cycle to avoid potential differences, even though the phase of the menstrual cycle does not seem to affect the $\dot{V}O_2$ kinetics response (Mattu et al. 2020). The mid-follicular phase was self-determined.

Visit 1: Ramp incremental cycling test to exhaustion. The test started with an initial workload of 10 W for 2 min (i.e. ‘unloaded’ cycling). This was followed by 10-15 W/min increments in power output for women or 15-20 W/min increments for men based on participants’ activity levels. Pedalling rate was held constant at an individually selected cadence between 60-75 revolutions per minute (rpm) and was maintained throughout all further testing. Failure in a test was determined as a drop in cadence exceeding 10 rpm for >5 s. Peak workload was the power output achieved at the point of failure. $\dot{V}O_{2peak}$ was the highest $\dot{V}O_2$ value (15-s average) attained during the test. The first ventilatory threshold (VT) was determined by visual inspection as the $\dot{V}O_2$ at which $\dot{V}_E/\dot{V}O_2$ exhibited a systematic non-linear increase without a concomitant increase in $\dot{V}_E/\dot{V}CO_2$ and the deflection point of $\dot{V}CO_2$ vs. $\dot{V}O_2$ (V-slope method) during the ramp incremental test (Beaver et al. 1986). The respiratory compensation point (RCP) was estimated by identifying the second non-linear increase of \dot{V}_E and $\dot{V}CO_2$, whereby an increase in $\dot{V}_E/\dot{V}O_2$ is accompanied by an increase of $\dot{V}_E/\dot{V}CO_2$ (Wasserman and McIlroy 1964).

Visit 2: Priming effect on high-intensity work-to-work cycling exercise. All participants performed four separate w-to-w transitions to constant-load high-intensity cycling at 50% delta ($\Delta 50\%$; the sum of the power output at VT and 50% of the difference between the power output at VT and $\dot{V}O_{2peak}$ obtained during the ramp incremental test) each commencing from an elevated baseline of 80% VT (80% of each participant’s VT). For all participants the power output at $\Delta 50\%$ was higher or the same than at RCP (see results). Given that in the present study the mean response times of $\dot{V}O_2$ during the ramp cycle exercise (Keir et al. 2018) were not accounted for when calculating these target power outputs, it is likely that power outputs at RCP (and at VT) were slightly overestimated. Thus, it is reasonable to assume that the $\Delta 50\%$ intensity for participants in the present study was within the lower region of the severe intensity domain (> critical power). The order of these bouts was fixed for all participants (Fig 1). Each transition consisted of 3 min of “unloaded” cycling at 10W, immediately followed by 6 min of moderate-intensity (80% VT) cycling which in turn was immediately followed by 6 min of high-intensity ($\Delta 50\%$) cycling. Two of these w-to-w transitions were completed without PE (unprimed w-to-w) and two bouts were undertaken preceded by a bout of PE (primed w-to-w). The unprimed w-to-w bout was used as PE. A pilot study carried out in our laboratory in young control individuals ($n = 6$) demonstrated that when a w-to-w bout was used as PE, its effect on subsequent w-to-w $\dot{V}O_2$ and [HHb+Mb] kinetics was not different compared with a condition where a

single 6-min 50% Δ bout was used as PE. Exercise was performed continuously with changes in power output initiated as a step function without giving prior warning to the individual. There was a 12 min rest period between each of the cycling bouts, except following the first primed w-to-w bout where participants remained seated in a chair for 45 min. This resting period has been shown to be sufficient for physiological parameters to return to baseline levels, and therefore, not to influence $\dot{V}O_2$ kinetics responses during subsequent exercise (Burnley et al. 2006). Eight participants (4 from each group) failed to complete 6 min of exercise at $\Delta 50\%$ during the w-to-w bouts in the unprimed condition, so only physiological responses collected over the same period (i.e., <6 min, range 2.5 – 5 min) during the unprimed and primed conditions were analysed. Heart rate (HR), gas exchange/ventilatory variables, muscle oxygenation & deoxygenation and muscle EMG were continuously measured during each cycling bout.

Measurements

During exercise, participants wore a facemask to continuously collect expired air using an online metabolic system (Innocor, Innovision A/S, Odense, Denmark) that measured airflow using a pneumotachometer. Carbon dioxide analysis was performed by using a photoacoustic gas analyser and oxygen was analysed using an oxygen sensor (Oxigraf Inc., USA) based on the principle of laser diode absorption spectroscopy. The system was calibrated prior to each test as per manufacturer's recommendations. Both the oxygen sensor and photoacoustic gas analyser require multi-point calibration that is routinely performed by the manufacturer every 6-12 months. Analysis of expired air allowed determination of pulmonary O_2 uptake ($\dot{V}O_2$), CO_2 output ($\dot{V}CO_2$), minute ventilation (\dot{V}_E) and the respiratory exchange ratio breath-by-breath. HR was recorded every 5 s (Polar S610i, Polar Ltd, Finland), with peak HR defined as the highest HR attained within the last 15 s prior to termination of the test.

A continuous wave NIRS system (Hamamatsu Niro 200Nx; Hamamatsu Photonics, Hamamatsu, Japan), was used to determine muscle oxygenation status non-invasively through the spatially resolved spectroscopy technique and modified Beer-Lambert principle with three wavelengths of emitting light ($\lambda = 735, 810, \text{ and } 850 \text{ nm}$). The theoretical basis of NIRS and its use in exercise measurements have been described in detail elsewhere (Ferrari et al. 2011) but briefly, this technique estimates the optical density changes of oxygenated (O_2Hb+Mb) and deoxygenated haemoglobin and myoglobin ($HHb+Mb$) based on the oxygen dependency of absorption changes for near-infrared light in these proteins. As the vastus lateralis (VL) muscle is a dominant locomotor muscle during cycling, the present study examined the concentration of $HHb+Mb$ ($\Delta[HHb+Mb]$), and tissue oxygenation index

(TOI) of the right vastus lateralis (VL) muscle. After shaving, cleaning and drying the skin, the probes were placed on the belly of the muscle, 5-8 cm above the lateral femoral condyle, parallel to the major axis of the thigh with a 3 cm spacing between the emitter and receiver. The probes were housed in a black rubber holder and secured on the skin surface with bi-adhesive tape and then covered with a dark elastic bandage, which minimised extraneous movement and the intrusion of stray light throughout the exercise protocol. Since the depth of the measured area was estimated to be approximately one-half the distance between the emitter and the receiver (~1.5 cm), the present study determined the thickness of the skin and adipose tissue at the site of the probe placement via 2D ultrasound operating in B-mode (Zonare Ultra Smart Cart, Software version 4.7, USA), to ensure that data largely represented absorption of near-infrared light in muscle tissue and not in subcutaneous fat. Individuals presenting with adiposity >1.5 cm over the site of interrogation on the vastus lateralis were excluded from the study.

Neuromuscular activity of the vastus lateralis muscle of the left leg was measured using surface electromyography (EMG). The area of the belly of the muscle was shaved and cleaned using a sterile alcohol wipe and the electrodes were placed in a bipolar Ag/AgCl arrangement 25mm apart (centre to centre) and in a plane which was estimated to be parallel to the direction of the muscle shortening during contraction, while a third ground electrode was placed on the left hip. The electrodes were taped in place and covered in a cloth bandage to prevent excessive movement during exercise. The EMG signal was measured using a Powerlab 26T (AD instruments, Sydney, Australia) at a sampling frequency of 1,000 Hz. All raw EMG data were demeaned and band passed filtered between 20 and 500 Hz with the filtered data then used to calculate integrated EMG (iEMG). Filtered data was rectified and then integrated for every 50 ms of EMG activity. The iEMG data were averaged in 15 s intervals throughout exercise, with these values normalized to the average measured during 15–165 s of unloaded cycling before the initial transition. Therefore, all iEMG data were calculated as a percentage of the initial unloaded cycling phase. Data from repeat trials were averaged, and iEMG at the time point equivalent to the onset of the $\dot{V}O_2$ slow component (TD_s , see *data analysis*) (the 15 s interval before the TD_s time point) and at end exercise (last 15 s of exercise) were calculated. $\Delta iEMG_{end-TD_s}$ was calculated as the difference between iEMG values at end-exercise and at the time point equivalent to the onset of TD_s . The EMG recordings were re-started prior to each w-to-w transition (i.e. upon initiation of the 3 min “unloaded” cycling) and were continuously measured for the duration of each w-to-w transition.

Data analysis

$\dot{V}O_2$ Kinetics: The breath-by-breath $\dot{V}O_2$ data for each transition were linearly interpolated to provide second-by-second values and time aligned such that time 0 represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, average response for each individual and further time-averaged into 5 s bins. During the moderate-intensity bouts, 5 participants (2 T2D and 3 ND during both conditions) revealed a small $\dot{V}O_2$ slow component suggesting that the power outputs in these participants were slightly above their VT. This was likely because the mean response times of $\dot{V}O_2$ during the ramp cycle exercise were not accounted for when calculating the target power outputs (Keir et al. 2018). Thus, averaged and smoothed responses for each participant for moderate-intensity exercise were fitted to either a monoexponential (*Eq. 1*) or biexponential (*Eq. 2*) function, while for high-intensity exercise responses were fitted to a biexponential function

$$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p [1 - e^{-(t-TD_p)/\tau_p}] F1 \quad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p [1 - e^{-(t-TD_p)/\tau_p}] F1 + A_s [1 - e^{-(t-TD_s)/\tau_s}] F2 \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t ; $\dot{V}O_2$ baseline (for moderate-intensity, in *Eq.*'s 1 & 2) is the mean $\dot{V}O_2$ in the final 30 s of unloaded cycling, whereas $\dot{V}O_2$ baseline (for high-intensity, in *Eq. 2*) is the mean $\dot{V}O_2$ in the final 60 s of the moderate-intensity cycling exercise preceding the step transition to high-intensity cycling exercise; A_p and A_s are the amplitudes of the increase in $\dot{V}O_2$ for the primary and slow component phases; TD_p and TD_s are the time delays of these phases, and τ_p and τ_s are the time constants of the phases, defined as the duration of time for which $\dot{V}O_2$ increases to a value equivalent to 63% of the amplitude. The conditional expressions F1 and F2 limit the fitting of the phase to the period at and beyond the time delay associated with that phase. The first 20 s of data after the onset of exercise (i.e., the phase I $\dot{V}O_2$ response) were deleted, while still allowing TD to vary freely (to optimize accuracy of parameter estimates (Murias et al. 2011)). The MRT was calculated through the fitting of a monoexponential curve to provide information on the “overall” $\dot{V}O_2$ kinetics during the high-intensity exercise bout, with no distinction made for the various phases of the response. The $\dot{V}O_2$ data were fit using a weighted least-squares non-linear regression procedure (TableCurve 2D, Systat, USA). Data points lying outside the 95% prediction interval during the initial fit of a model were excluded. For moderate-intensity exercise only estimates representing the primary phase are presented. Whilst the presence of a slow component was detected in 5 participants during the moderate-intensity bouts, the presence of this phase does not appear to significantly affect the parameter estimates of the earlier phases (Wilkerson et al. 2004). The end-exercise $\dot{V}O_2$ response, referred to as End A, was calculated as the averaged $\dot{V}O_2$ over the last 30 s. Because the

asymptomatic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the slow component was calculated as the absolute difference between the End A and $\dot{V}O_2$ baseline + A_p . The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response [i.e. $A_s / (A_p + A_s)$]. The functional “gain” of the primary $\dot{V}O_2$ response (G_p) was calculated as the difference between $\dot{V}O_2$ A_p and $\dot{V}O_2$ baseline normalized to the difference in power outputs between the moderate-intensity exercise and unloaded cycling; and the functional gain of the entire response at the end of the high-intensity exercise bout (i.e. end-exercise gain) was calculated in a similar manner.

[HHb+Mb] kinetics and TOI. To provide information on muscle deoxygenation throughout the protocol, we modelled the [HHb+Mb] response for moderate- and high-intensity exercise, fitting the data to either a monoexponential (Eq. 1) or biexponential (Eq. 2) function (see above). In the moderate-intensity exercise the 5 participants who showed a small $\dot{V}O_2$ slow component also showed a [HHb+Mb] slow component, so, for these participants data were fitted using a biexponential function, but only estimates representing the primary phase are presented. As per the $\dot{V}O_2$ data, the NIRS-derived Δ [HHb+Mb] data for each transition were linearly interpolated to provide second-by-second values and time aligned. Data from each transition were ensemble-averaged to yield a single average response for each individual, and further time-averaged into 5 s bins. A time delay (TD) at the onset of exercise occurs in the [HHb+Mb] profile before it increases with an exponential like time course (DeLorey et al. 2003) which has been interpreted to reflect a tight coupling between muscle O_2 uptake and local O_2 delivery (DeLorey et al. 2003). This was determined in the present study via visual inspection as a systematic increase above the pre-transition level. [HHb+Mb] data were fitted from the end of this TD to the end of the exercise bout. For the moderate- and high-intensity exercise, the time course for the primary phase of the Δ [HHb+Mb] response, referred to as the effective response time ($\tau' \Delta$ [HHb+Mb]), was determined from the sum of the TD and τ from the onset of exercise. TOI was determined at baseline (30 s prior to each transition to the moderate-intensity domain), at every minute during the moderate-intensity cycling exercise; at min 1 and 2 into the high-intensity exercise transition (15 s bins centred on every 60 s), and at the end exercise (final 30 s) to allow comparisons between conditions in all participants.

Statistical analysis

Prior to analysis, normal distribution was assessed using the Shapiro-Wilk’s test. Physical characteristics and physiological responses derived from the ramp test between groups were compared using the unpaired Student’s

t-test for parametric analyses, or the Mann-Whitney U test for non-parametric analyses. The kinetics parameter estimates for $\dot{V}O_2$ and [HHb+Mb], and $\Delta iEMG_{end-TDs}$ responses were analysed by using a two-way repeated measures ANOVA [condition (unprimed, primed) x diabetes status (T2D, ND)] and the post hoc Tukey test. TOI responses at different time points within the w-to-w transitions were compared using a 3-way repeated measures ANOVA (time x condition x diabetes status). Finally, correlations between PE-induced absolute changes in $\dot{V}O_2 A_s$ and $\Delta iEMG_{end-TDs}$ were established using the Pearson product-moment correlation coefficient (Pearson r). A power analysis indicated that 9 participants per group were required to detect a PE-induced reduction of ~30% in $\dot{V}O_2 \tau_p$ during the w-to-w transitions (primary outcome) with a power of 0.80 and alpha of 0.05. This was based on previously published data on the effect of PE on subsequent $\dot{V}O_2 \tau_p$ during cycling w-to-w transitions in the supine posture (i.e. when O_2 delivery to the active muscles was reduced at the outset) (DiMenna et al. 2010b). Statistical significance was accepted as $P < 0.05$. All values are expressed as mean \pm standard deviation (SD) or as median and interquartile ranges for data that were deemed not normally distributed.

Results

Physical characteristics and activity levels.

Participants' physical characteristics are presented in Table 1. Both groups were well matched according to sex, age, body mass, body mass index and activity levels. As expected, participants with T2D displayed higher HbA_{1c} and fasting plasma glucose levels.

Performance data from ramp incremental cycling test

Absolute $\dot{V}O_{2peak}$ (T2D: 1.94 ± 0.53 L.min⁻¹; ND: 2.47 ± 0.54 L.min⁻¹; $P = 0.049$) and $\dot{V}O_{2peak}$ normalised to body mass (T2D: 22.4 ± 4.3 mL.kg⁻¹.min⁻¹; ND: 29.7 ± 7.7 mL.kg⁻¹.min⁻¹; $P = 0.012$) were significantly reduced in individuals with T2D compared with healthy controls while peak power output tended to be lower in T2D (T2D: 149 ± 45 W; ND: 192 ± 57 W; $P = 0.092$). The power outputs equivalent to 80% VT were lower in T2D (T2D: 64 ± 17 W; ND: 96 ± 44 W; $P = 0.043$) while power outputs equivalent to $\Delta 50\%$ (T2D: 116 ± 33 W; ND: 158 ± 58 W; $P = 0.076$) and RCP (T2D: 112 ± 33 W; ND: 153 ± 55 W; $P = 0.073$) showed a tendency to be reduced in diabetes.

Effect of PE on $\dot{V}O_2$ kinetics, EMG and NIRS-derived responses during high-intensity exercise of the w-to-w transition

$\dot{V}O_2$ kinetics. The parameter estimates of the $\dot{V}O_2$ kinetics response for the high-intensity exercise bouts with and without a prior PE are presented in Table 2, and responses for representative individuals are shown in Fig 2. In the unprimed transition the $\dot{V}O_2$ τ_p and overall $\dot{V}O_2$ MRT were significantly ($P = 0.035$ & $P = 0.049$ respectively) longer in T2D compared with controls. PE resulted in a significant reduction in the $\dot{V}O_2$ MRT in both groups, while $\dot{V}O_2$ τ_p values were also reduced following PE in T2D ($P = 0.001$) but not in controls ($P = 0.24$). Subsequent to PE $\dot{V}O_2$ A_s was reduced in both groups ($P = 0.001$) while $\dot{V}O_2$ A_p was elevated (main effect, priming condition, $P = 0.015$).

$\Delta[HHb+Mb]$ kinetics. Kinetics parameters for $\Delta[HHb+Mb]$ as well as TOI baseline & amplitude values are displayed in Table 3 while TOI values during the w-to-w transitions are shown in Fig 3. In the unprimed condition, the parameter estimates for the $[HHb+Mb]$ kinetics responses were similar between groups. PE induced a reduction in the $\Delta[HHb+Mb]$ A_p in both groups (main effect, priming condition, $P = 0.004$), but it did not affect the effective response time of the $\Delta[HHb+Mb]$ response in either group. TOI values were higher during the primed high-intensity exercise bout in both groups (main effect, priming condition, $P = 0.002$). The magnitude of the change in TOI from baseline to end-exercise was not affected by prior PE. Participants with T2D showed lower TOI than controls (main effect, diabetes status, $P = <0.001$).

EMG. Representative iEMG responses during the w-to-w transitions are shown in Fig 4, while relative iEMG responses between the time points equivalent to end-exercise and the onset of $\dot{V}O_2$ slow component are shown in Fig 5. The $\Delta iEMG_{\text{end-TDs}}$ was significantly reduced subsequent to PE in both groups (main effect, priming condition, $P = <0.001$) (T2D unprimed: 22 ± 18 %, T2D primed: 1 ± 10 %; controls unprimed: 30 ± 37 %, controls primed: 3 ± 20 %). Absolute changes in $\dot{V}O_2$ A_s and $\Delta iEMG_{\text{end-TDs}}$ from unprimed to primed conditions were not correlated in controls ($r = 0.09$, $P = 0.85$), or among individuals with T2D ($r = 0.49$, $P = 0.22$).

Effect of PE on $\dot{V}O_2$ kinetics and NIRS-derived responses at moderate-intensity exercise of the w-to-w transition

The parameter estimates of the $\dot{V}O_2$ kinetics response for the moderate-intensity exercise bouts are presented in Table 2. In both, the unprimed and primed conditions $\dot{V}O_2$ τ_p was significantly slower in T2D compared with controls (main effect, group, $P = 0.016$), while PE accelerated $\dot{V}O_2$ τ_p in both groups (main effect, priming condition, $P = 0.007$). Kinetics parameters for $\Delta[HHb+Mb]$ are displayed in Table 3. Parameter estimates were similar between groups in the unprimed condition. PE did not affect the amplitude or the effective response time of the $\Delta[HHb+Mb]$ response in either group. TOI responses were higher during the primed moderate-intensity

exercise bout in both groups (Fig 3 & Table 3). In addition, the magnitude of the change in TOI from baseline to end-exercise was larger following PE in both groups (main effect, priming condition, $P < 0.001$).

Discussion

To our knowledge this is the first study to explore the influence of PE on the temporal relationship between the adaptation of muscle O_2 consumption and delivery during high-intensity cycling initiated from a moderate-intensity baseline in T2D. In agreement with our primary hypothesis, PE reduced $\dot{V}O_2 \tau_p$ during the high-intensity cycling bout of the w-to-w transition in T2D in the absence of significant changes in the dynamic response of $\Delta[HHb+Mb]$. Additionally, consistent with our second hypothesis, PE significantly reduced the $\dot{V}O_2 A_s$ during the high-intensity exercise bout, accompanied with a reduction in muscle electromyographic activity between the end-exercise and the time point equivalent to the onset of $\dot{V}O_2$ slow component. Together, these priming effects resulted in a reduction in the MRT of the overall $\dot{V}O_2$ response.

Effect of PE on $\dot{V}O_2 \tau_p$ during high-intensity exercise of the w-to-w transition

In the present study, PE did not significantly reduce $\dot{V}O_2 \tau_p$ during the subsequent high-intensity bout of the w-to-w transition among ND participants; and these findings are consistent to those observed during unprimed and primed upright severe-intensity w-to-w transitions (~ 42 vs. ~ 42 s respectively) in healthy individuals (DiMenna et al. 2008). Given that PE facilitates convective and diffusive muscle O_2 delivery, (Gerbino et al. 1996; Sahlin et al. 2005; Jones et al. 2006), our findings, and those by DiMenna et al (DiMenna et al. 2010b), suggest that the $\dot{V}O_2 \tau_p$ responses in the control condition were not impaired by O_2 delivery limitation. In contrast, for T2D, $\dot{V}O_2 \tau_p$ responses during w-to-w transitions following PE were significantly reduced ($\sim 36\%$ reduction) bringing the $\dot{V}O_2 \tau_p$ in T2D on a par with control counterparts (~ 37 s). This effect was also evidenced in healthy participants during severe-intensity cycling w-to-w transitions in the supine position (DiMenna et al. 2010b), thus, compromising exercising muscle perfusion pressure and O_2 delivery (Egaña and Green 2005, 2007). Specifically, PE subsequently induced a significant reduction in the lengthened $\dot{V}O_2 \tau_p$ during the supine posture, aligning it with that observed in the unprimed upright posture. This was likely by negating the constrained O_2 delivery, attributed to a loss of gravity-enhanced perfusion pressure in the active muscles (Jones et al. 2006; Egaña et al. 2013; Egaña et al. 2010a; Egaña et al. 2010b).

Given that the impaired $\dot{V}O_2 \tau_p$ in T2D appears to be mediated, at least in part by limitations in O_2 supply to contracting muscle (Kiely et al. 2014; Bauer et al. 2007; MacAnaney et al. 2011), it is likely that the priming-induced speeding in $\dot{V}O_2 \tau_p$ in T2D herein was elicited by an enhanced O_2 supply. The increased O_2 availability at exercise onset in the primed exercise bout, evidenced by the elevated TOI further substantiates this notion. It is likely that this was mediated by a PE-induced greater vasodilation and muscle blood flow at the onset of exercise (Hughson et al. 2003; Gerbino et al. 1996) and increased lactic acidosis, via an enhanced blood-to-myocyte O_2 diffusion gradient through a rightward shift of the oxyhaemoglobin dissociation curve (Boning et al. 1991; Wasserman et al. 1991); even if this effect is not apparent following prior arm cranking exercise (Fukuba et al. 2002). However, we cannot exclude the possibility that the priming-augmented $\dot{V}O_2 \tau_p$ observed herein, was also partially mediated by the upregulation of rate-limiting mitochondrial oxidative enzymes (Gurd et al. 2006, 2009).

Effect of PE on $\dot{V}O_2 A_s$ and iEMG during high-intensity exercise of the w-to-w transition

In the present study, in addition to decreasing $\dot{V}O_2 \tau_p$, PE significantly reduced the amplitude of the $\dot{V}O_2$ slow component during the high-intensity bout of the w-to-w transition in participants with T2D. In addition, despite PE not influencing $\dot{V}O_2 \tau_p$ in the controls, PE reduced the $\dot{V}O_2 A_s$ during the high-intensity bout, thus, shortening the overall MRT of the $\dot{V}O_2$ response. These PE-induced reductions in the $\dot{V}O_2 A_s$, without altering $\dot{V}O_2 \tau_p$ in healthy controls are in accordance with the literature centred on the influence of PE on heavy/severe-intensity upright cycle exercise, both, from an elevated and an unloaded baseline (Burnley et al. 2006; Jones et al. 2008; Jones et al. 2006; Scheuermann et al. 2001; Wilkerson and Jones 2007; Goulding et al. 2017; Burnley et al. 2000; Fukuba et al. 2002); however, the governing mechanisms remain to be elucidated.

One such mechanism relates to priming-induced changes in the motor unit recruitment pattern. In this regard, in the present study, the difference in iEMG between end-exercise and the time point equivalent to the onset of $\dot{V}O_2 A_s$ ($\Delta iEMG_{end-TDs}$) in the unprimed bout was significantly reduced following PE in both groups. Our findings are consistent with reductions in $\Delta iEMG$ between end-exercise and min 2 during primed compared with unprimed upright severe-intensity w-to-w cycling transitions in young active participants (DiMenna et al. 2008). Given the transition to high-intensity exercise from an elevated baseline would mandate the recruitment of predominantly type II muscle fibres, it is plausible that PE elicited a reduction in the requirement for additional type II muscle fibre activation as the exercise proceeded, and as such, the associated $\dot{V}O_2$ cost of that activation was reduced (DiMenna et al. 2008). Further extending this notion, DiMenna and colleagues (Dimenna et al. 2010a)

demonstrated a PE-induced reductions in the amplitudes of the [PCr] and $\dot{V}O_2$ slow components (50% and 46% respectively) during prone knee-extension w-to-w transitions concomitant with a blunting of the $\Delta iEMG$. A reduction in the recruitment of these less efficient muscle fibres could serve to dampen the increase in the sustained metabolic acidosis, deemed a likely driving force behind the slow components of both [PCr] and $\dot{V}O_2$, (Rossiter et al. 2002; Krstrup et al. 2004). The combined iEMG and tissue oxygenation data in the present study may also suggest a priming-enhanced distribution of intramuscular blood flow. Consequently, the anaerobic contribution would decrease, precluding the recruitment of additional motor units, whilst favouring a more homogenous pool of highly oxidative type I muscle fibres (DiMenna et al. 2010b). By the same token, we cannot negate the upregulation of enzymatic processes within the type I fibres already recruited, improving the metabolic stability within. Subsequently, a smaller reduction in [PCr] and Gibbs free energy of ATP hydrolysis, as well as a smaller increase in [Pi] and [ADP] are ensured, thus sparing the activation of type I motor units herein.

Given that PE herein facilitated a reduction in the $\dot{V}O_2 A_s$ of the severe-intensity w-to-w transition in individuals with T2D, combined with a reduction in $\Delta iEMG_{end-TDs}$ of that same bout, it is likely that the priming-induced reduction in $\dot{V}O_2 A_s$ herein may also be related to modified motor unit recruitment patterns. However, in addition, given that type II fibres operate at a lower microvascular PO_2 , the priming-enhanced O_2 delivery plausibly increased the blood-to-myocyte flux and thus intramyocyte PO_2 . This is all the more pertinent considering an altered muscle fibre distribution has been evidenced in individuals with T2D (Marin et al. 1994) showing increased proportions in type IIb fibres (Mogensen et al. 2007). However, it should be noted that given the variability associated with measurement and normalisation of iEMG, some previous studies do not support the association between neuromuscular activation and the $\dot{V}O_2$ slow component (Scheuermann et al. 2001). In addition, we did not observe a significant correlation between PE-induced absolute reductions in $\Delta iEMG_{end-TDs}$ with reductions in A_s .

Effect of PE on $\dot{V}O_2 \tau_p$ during moderate-intensity exercise of the w-to-w transition

During the unprimed moderate-intensity cycling bout and in line with previous findings (reviewed by Green et al (Green et al. 2015)), individuals with T2D displayed a significantly longer $\dot{V}O_2 \tau_p$ than their healthy counterparts (~35 vs. ~44 s, respectively). Subsequent to PE both groups demonstrated similar reductions in the $\dot{V}O_2 \tau_p$, consistent with recent findings from our group in a larger number of middle-aged individuals with T2D (Rocha et al. 2019), and in several previous studies involving young and older untrained healthy individuals presenting with

initially slow $\dot{V}O_2$ τ_p (DeLorey et al. 2004; Gurd et al. 2005; De Roia et al. 2012). NIRS-derived overall muscle deoxygenation kinetics (τ' [HHb+Mb]) herein, were not affected by PE in any of the groups; therefore, it is likely that the speeding of the $\dot{V}O_2$ kinetics response was attributed to a better matching of microvascular O_2 delivery to utilisation.

Limitations

While a subset of participants (4 in each group) did not complete the required 6 min of high-intensity cycling exercise during the w-to-w transitions, we believe this had little influence on the interpretation of our findings given that the majority (8 in each group) completed at least 4 min of the bout and showed a clear $\dot{V}O_2$ slow component phase. Although the current protocol did not allow the random assignment of unprimed and primed conditions, this likely has a small impact on the results given that the sequence of the exercise transitions was the same for all participants. We acknowledge the NIRS-derived oxygenation and deoxygenation data was limited to one superficial muscle. Thus, the structural and functional heterogeneity extant within individual muscles, in particular relating to vascularity and fibre type, fibre recruitment, vascular control, and blood flow (Koga et al. 2011; McDonough et al. 2005), in addition to variances identified both between muscles and within deep and superficial muscle segments (Okushima et al. 2015; Saitoh et al. 2009), warrant consideration. Additionally, 3 participants with T2D were classified as hypertensive and also had hyperlipidaemia; whereas all controls were normotensive, with one presenting with hyperlipidaemia. Further studies are needed to better establish if the higher rates of hypertension and/or hyperlipidaemia observed within the T2D group in the present study may have any significant impact on the findings presented herein.

Conclusions

The present study primarily demonstrated that priming exercise accelerates the primary time constant of $\dot{V}O_2$ during high-intensity w-to-w transitions in middle-aged individuals with T2D. This effect was likely mediated by a priming-induced increase in O_2 delivery within the microvasculature of the working muscle, serving to alleviate the metabolic strain to maintain $\dot{V}O_2$. In addition, PE decreased the amplitude of the $\dot{V}O_2$ slow component which was likely influenced by an augmented motor unit recruitment pattern. Thus, from a physiological perspective the combination of a PE intervention with the w-to-w model helps expand the insight that the impaired $\dot{V}O_2$ kinetics in T2D are influenced by limitations in O_2 delivery. From a practical perspective, employing the work-to-work protocol is of great relevance as it replicates metabolic transitions from light to higher metabolic rates akin to

those in daily life. Given individuals with T2D perceive light to moderate exercise as being more difficult than healthy counterparts (Huebschmann et al. 2009), a more sedentary lifestyle is likely, which is independently associated with worsening of cardiovascular outcomes in this burgeoning population. Therefore, the potential that lies within an acute intervention such as priming or warm-up exercise which serves to heighten the oxidative capacity of muscles and increase the therapeutic effect of exercise warrants further recognition.

Author contributions

N.G., J.R., M.E., D.O'S. and S.G. contributed to the study conception and design. N.G. and J.R. performed data collection. N.G., and M.E. analysed data. N.G. and M.E. drafted the manuscript. S.G., D.O'S and J.R. contributed to critically revising of this manuscript. All authors approved the final version.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest, financial or otherwise.

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Figure legends

Figure 1

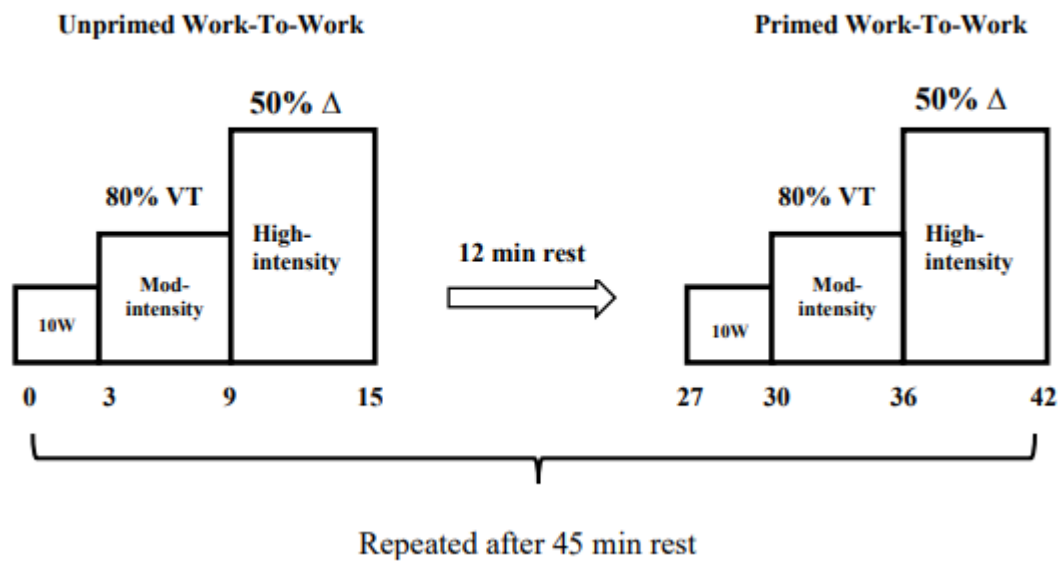


Fig 1 Schematic representation of the protocol. Unprimed and primed work-to-work cycling step transitions performed at high-intensity cycling exercise ($\Delta 50\%$; the sum of the power output at VT and 50% of the difference between the power output at VT and $\dot{V}O_{2peak}$), each commencing from an elevated baseline of moderate-intensity (power output corresponding to 80% of each participant's first ventilatory threshold, VT). All step transitions, each lasting 6 min, were preceded by 3 min of cycling at 10 W (i.e. 'baseline' cycling). Unprimed and primed work-to-work transitions were separated by 12 min of passive rest. The 2 step transitions (unprimed and primed work-to-work) were repeated following 45 min of passive rest within the same laboratory visit.

Figure 2

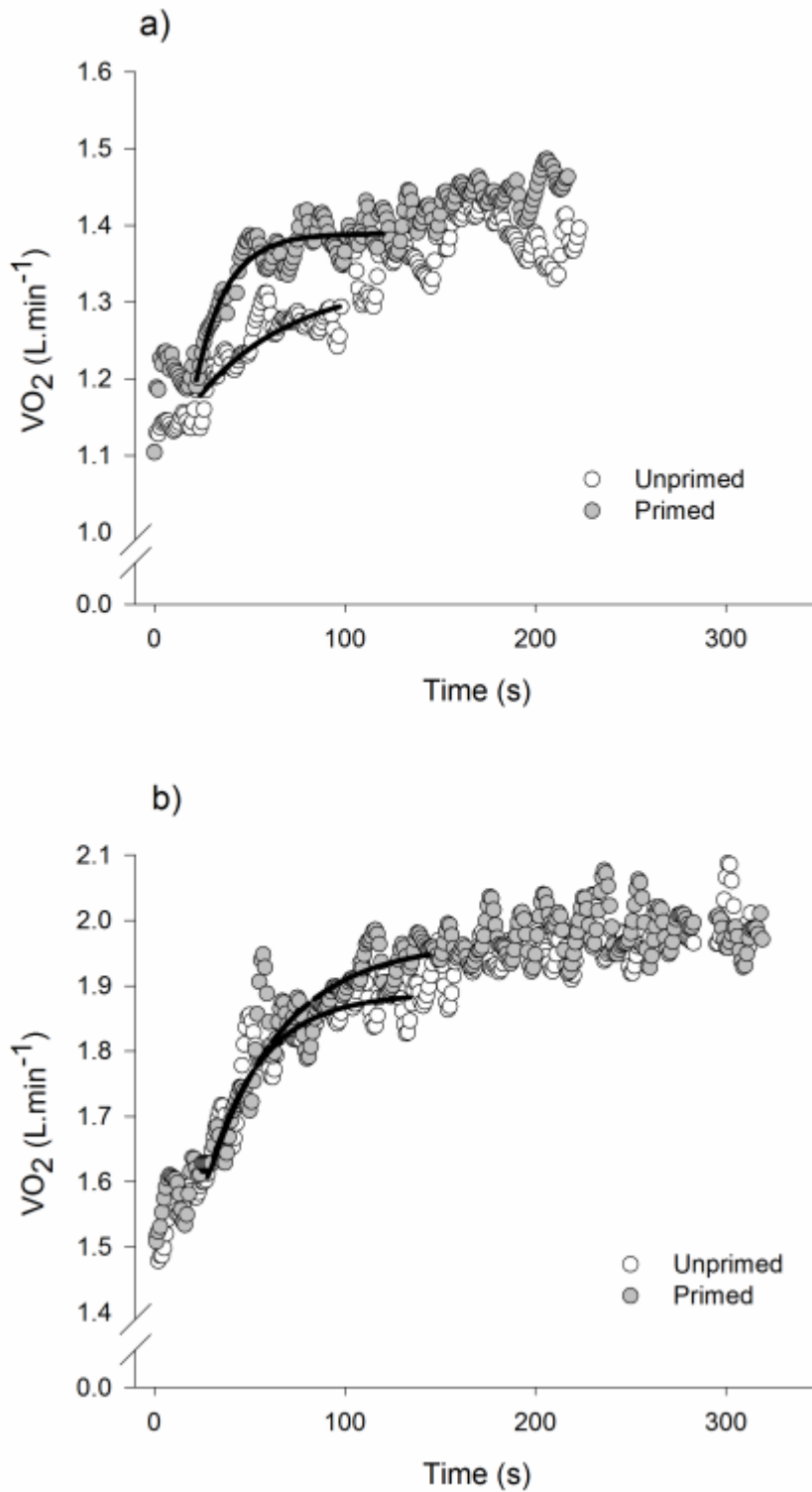
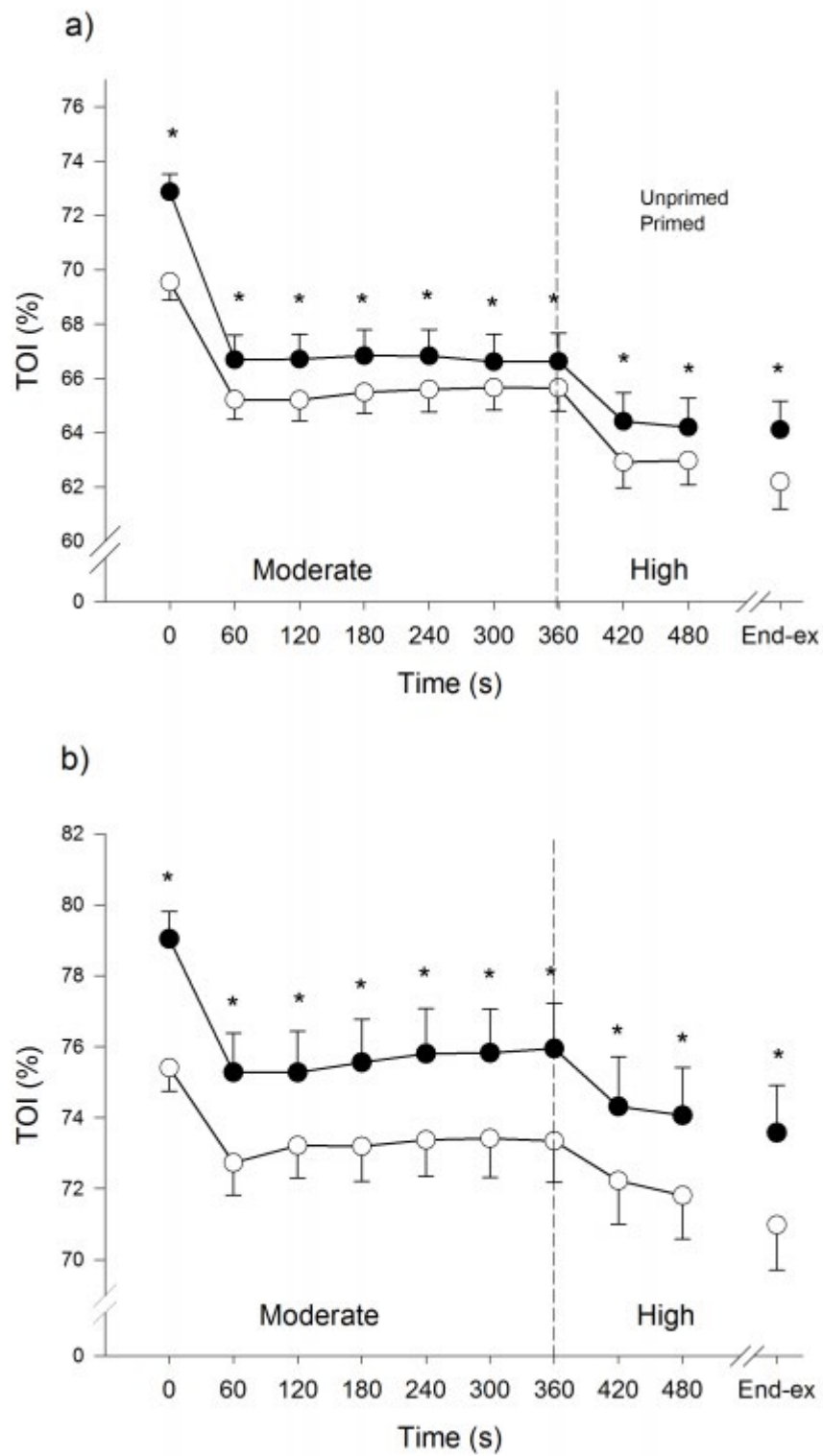


Fig 2. Oxygen uptake ($\dot{V}O_2$) responses for a representative individual with type 2 diabetes (A) and a healthy control (B) during high-intensity work-to-work cycling transitions without priming exercise (open circles) and with priming exercise (solid circles). The continuous lines of best fit illustrate the primary phase of the oxygen uptake ($\dot{V}O_2$) response. Note the relatively slower response of the primary phase of the $\dot{V}O_2$ response in the

730 unprimed compared with the primed bout in T2D.

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Figure 3



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Fig 3. Mean \pm SD total oxygenation index (TOI) at moderate and high-intensity exercise during the work-to-work transitions without priming exercise (open circles) and with priming exercise (solid circles) in T2D (A) and healthy controls (B). * $P < 0.05$ vs. unprimed within same diabetes status group (i.e. within controls or within Type 2 diabetes).

Figure 4

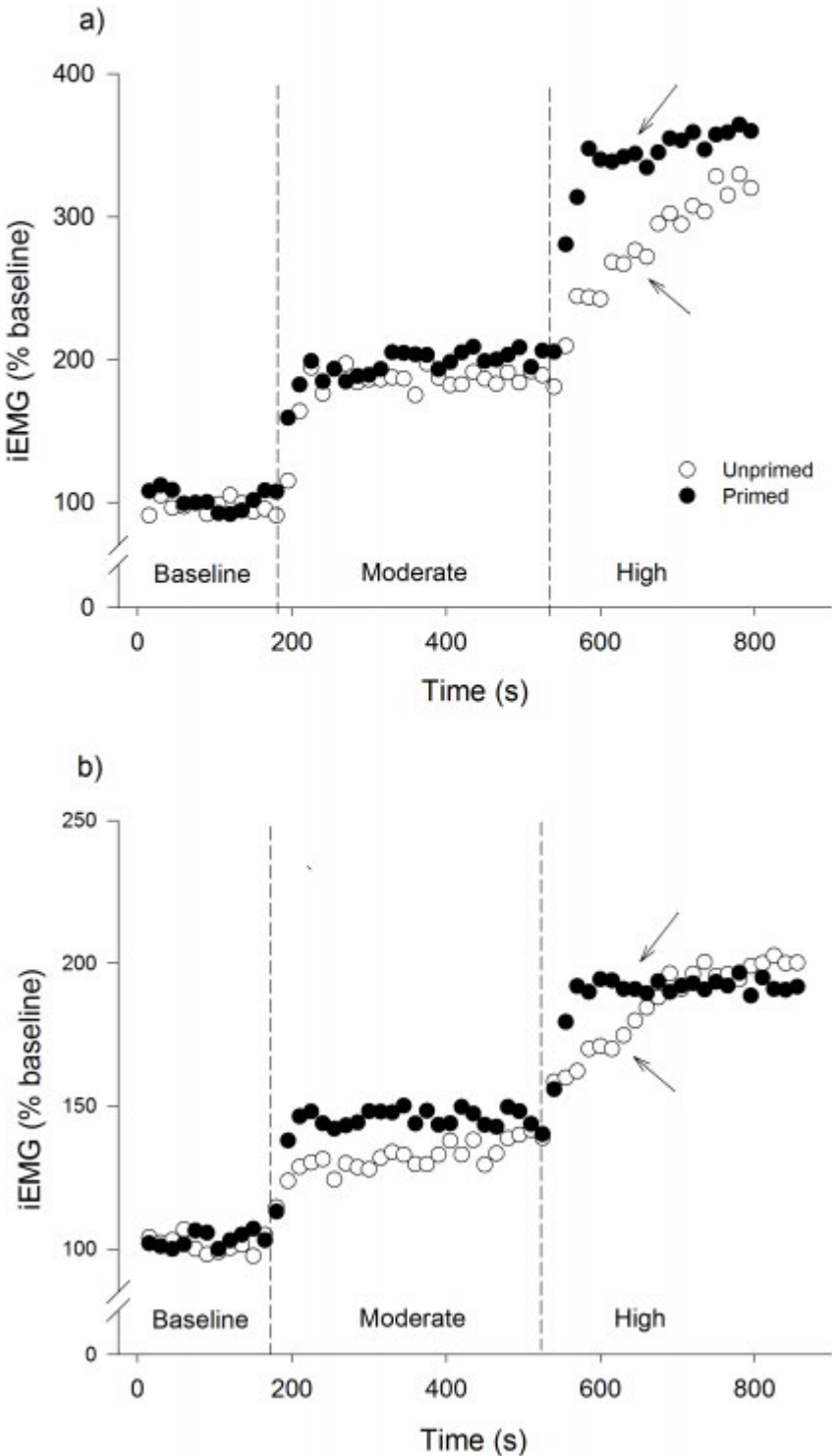


Fig 4: Integrated surface electromyographic (iEMG) responses for a representative individual with type 2 diabetes (A) and a healthy control (B) during moderate and high-intensity work-to-work cycling transitions without

priming exercise (open circles) and with priming exercise (solid circles). The arrows indicate the time point equivalent to the onset of the $\dot{V}O_2$ slow component.

Figure 5

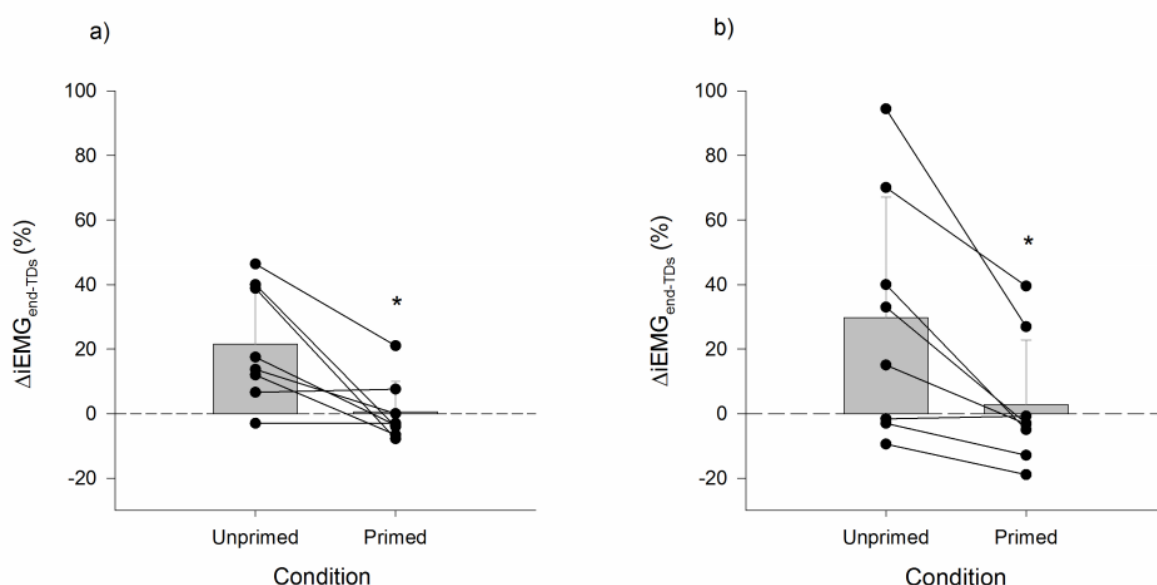


Fig 5: Individual and mean \pm SD (*bar graph*) changes in integrated surface electromyographic (iEMG) responses between end-exercise and the time point equivalent to the oxygen uptake slow component ($\dot{V}O_2$ TDs) ($\Delta iEMG_{end-TDs}$) during high-intensity work-to-work transitions without priming exercise (unprimed) and with priming exercise (primed) in T2D (A) and healthy controls (B). * $P < 0.05$ vs. unprimed within same diabetes status group (i.e. within controls or within Type 2 diabetes).

Table 1. Physical characteristics and activity levels.

	Controls	T2D	<i>P</i> Value
<i>n</i>	9	9	
Physical characteristics			
Sex (male, female), <i>n</i>	5, 4	5, 4	
Age, yr	45 \pm 12	48 \pm 9	0.48

Stature, m	1.67 ± 0.07	1.70 ± 0.08	0.46
BMI, kg/m ²	30 (4)	28 (8)	0.72
Body Mass, kg	82.0 (8.5)	79.0 (32.8)	0.57
Fat layer VL, mm	12.7 (10.2)	6.5 (2.8)	0.23
HbA1c, %	5.1 (0.2)*	6.9 (1.4)	0.02
FPG, mmol/L	4.4 ± 0.8*	7.2 ± 1.3	0.01
Time since diagnosis, yr		7.3 ± 4.0	
Total cholesterol, mmol/L	3.85 ± 0.88	4.50 ± 0.77	0.59
LDL-C, mmol/L	2.14 ± 0.86	2.43 ± 0.76	0.65
HDL-C, mmol/L	1.20 ± 0.17	1.00 ± 0.17	0.10
Triglycerides, mmol/L	1.12 ± 0.48	2.33 ± 1.29	0.07
Habitual physical activity			
Inactive, h/day	19.2 ± 1.7	18.3 ± 1.4	0.40
Light, h/day	3.8 ± 1.1	5.1 ± 1.3	0.13
Moderate, h/day	0.73 ± 0.50	0.48 ± 0.28	0.39
Vigorous, h/day	0.20 (0.25)	0.05 (0.33)	0.35

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Values are means ± SD for variables that were normally distributed and median with interquartile range in parentheses for variables which showed significant skewness and were not normally distributed in one or both groups. *n*, no. of participants. Some variables have missing values, and the sample sizes are as follows: fat layer vastus lateralis (VL), *n* = 7 [nondiabetic control (ND)] and 8 [type 2 diabetes (T2D)];

Table 2. Dynamic response characteristics of oxygen uptake ($\dot{V}O_2$) during moderate-intensity and high-intensity cycling exercise of the work-to-work transitions

	Unprimed		Primed	
	Controls	Type 2 diabetes	Controls	Type 2 diabetes
<i>N</i>	9	9	9	9
<i>Moderate-intensity</i>				
Baseline $\dot{V}O_2$, L/min	0.77 ± 0.13	0.89 ± 0.27	0.82 ± 0.11	0.90 ± 0.23
$\dot{V}O_2 A_p$, L/min	0.82 ± 0.44	0.50 ± 0.21	0.85 ± 0.51	0.51 ± 0.19
$\dot{V}O_2 \tau_p$, s	$34.6 \pm 7.3^\dagger$	43.8 ± 11.2	$25.6 \pm 7.7^\dagger^*$	$33.2 \pm 11.5^*$
CI ₉₅ $\dot{V}O_2 \tau_p$, s	4.4 ± 2.1	5.1 ± 1.9	4.0 ± 1.2	4.7 ± 0.8
$\dot{V}O_2$ end A, L/min	1.64 ± 0.54	1.44 ± 0.39	1.73 ± 0.65	1.43 ± 0.33
$\dot{V}O_2 G_p$, mL.min ⁻¹ .W ⁻¹	9.6 ± 1.7	9.4 ± 2.5	9.3 ± 2.3	9.5 ± 2.3
<i>High-intensity</i>				
Baseline $\dot{V}O_2$, L/min	1.64 ± 0.54	1.44 ± 0.39	1.73 ± 0.65	1.43 ± 0.33
$\dot{V}O_2 A_p$, L/min	$0.53 \pm 0.15^\dagger$	0.33 ± 0.12	$0.55 \pm 0.15^\dagger^*$	$0.40 \pm 0.16^*$
$\dot{V}O_2 \tau_p$, s	$43.6 \pm 9.8^\dagger$	58.6 ± 16.6	37.7 ± 6.9	$37.2 \pm 19.9^*$
CI ₉₅ $\dot{V}O_2 \tau_p$, s	8.8 ± 2.4	8.7 ± 2.9	9.0 ± 1.9	8.0 ± 2.8
$\dot{V}O_2 A_s$, L/min	0.21 ± 0.13	0.16 ± 0.09	$0.13 \pm 0.09^*$	$0.11 \pm 0.04^*$
$\dot{V}O_2 A_s$, %	27.5 ± 10.3	32.7 ± 11.0	$18.5 \pm 10.6^*$	$22.5 \pm 7.5^*$
$\dot{V}O_2 TD_s$, s	127 ± 47	119 ± 7	129 ± 50	106 ± 43
$\dot{V}O_2$ end A, L/min	2.37 ± 0.61	1.93 ± 0.50	2.41 ± 0.66	1.95 ± 0.50
$\dot{V}O_2$ MRT, s	$73 \pm 15^\dagger$	94 ± 31	$57 \pm 17^*$	$59 \pm 22^*$
CI ₉₅ $\dot{V}O_2$ MRT, s	7.4 ± 2.3	7.9 ± 1.4	7.1 ± 2.1	7.1 ± 2.5
End-exercise $\dot{V}O_2$ gain, mL.min ⁻¹ .W ⁻¹	11.2 ± 1.6	10.3 ± 1.7	11.0 ± 1.9	10.2 ± 1.7

Values are means \pm SD; *n* = no. of participants. A, amplitude; τ , time constant; end A, steady-state oxygen uptake ($\dot{V}O_2$) response; CI₉₅ 95% confidence interval; G, oxygen uptake ($\dot{V}O_2$) gain; TD, time delay; p, primary phase; s slow component phase.

* $P < 0.05$ vs. unprimed within same diabetes status group (i.e. within controls or within Type 2 diabetes). $^\dagger P < 0.05$ vs. participants with type 2 diabetes within same condition (i.e. within unprimed or primed).